The ISME Journal (2011) 5, 1634–1640
© 2011 International Society for Microbial Ecology All rights reserved 1751-7362/11
www.nature.com/ismej

ORIGINAL ARTICLE

Common ancestry of iron oxide- and iron-sulfide-based biomineralization in magnetotactic bacteria

Fernanda Abreu[1], Mauricio E Cantão[2,3], Marisa F Nicolás[2], Fernando G Barcellos[2], Viviana Morillo[1], Luiz GP Almeida[2], Fabrícia F do Nascimento[1], Christopher T Lefèvre[4], Dennis A Bazylinski[4], Ana Tereza R de Vasconcelos[2] and Ulysses Lins[1]

[1]*Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil;* [2]*Laboratório de Bioinformática, Laboratório Nacional de Computação Científica, Rio de Janeiro, Brasil;* [3]*Instituto Nacional de Metrologia, Normalização e Qualidade Industrial, Rio de Janeiro, Brasil and* [4]*School of Life Sciences, University of Nevada at Las Vegas, Las Vegas, NV, USA*

Magnetosomes are prokaryotic organelles produced by magnetotactic bacteria that consist of nanometer-sized magnetite (Fe_3O_4) or/and greigite (Fe_3S_4) magnetic crystals enveloped by a lipid bilayer membrane. In magnetite-producing magnetotactic bacteria, proteins present in the magnetosome membrane modulate biomineralization of the magnetite crystal. In these microorganisms, genes that encode for magnetosome membrane proteins as well as genes involved in the construction of the magnetite magnetosome chain, the *mam* and *mms* genes, are organized within a genomic island. However, partially because there are presently no greigite-producing magnetotactic bacteria in pure culture, little is known regarding the greigite biomineralization process in these organisms including whether similar genes are involved in the process. Here using culture-independent techniques, we now show that *mam* genes involved in the production of magnetite magnetosomes are also present in greigite-producing magnetotactic bacteria. This finding suggest that the biomineralization of magnetite and greigite did not have evolve independently (that is, magnetotaxis is polyphyletic) as once suggested. Instead, results presented here are consistent with a model in which the ability to biomineralize magnetosomes and the possession of the *mam* genes was acquired by bacteria from a common ancestor, that is, the magnetotactic trait is monophyletic.

The ISME Journal (2011) **5,** 1634–1640; doi:10.1038/ismej.2011.35; published online 21 April 2011
Subject Category: integrated genomics and post-genomics approaches in microbial ecology
Keywords: biomineralization evolution; greigite; magnetite; magnetotactic bacteria; magnetosome; horizontal gene transfer

Introduction

Magnetite-producing magnetotactic bacteria (MTB) are phylogenetically affiliated with the *Alphaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* classes of the *Proteobacteria* and the *Nitrospirae* phylum (Bazylinski and Frankel, 2004; Lefèvre *et al.*, 2010a). Greigite-producing MTB have not been cultured and include a group of morphologically similar multicellular magnetotactic prokaryotes affiliated with *Deltaproteobacteria* (Abreu *et al.*, 2007; Simmons and Edwards, 2007) and large rod-shaped bacteria (Pósfai *et al.*, 1998a), whose phylogeny has not been studied in detail. One report suggests that at least one type of greigite-producing MTB is affiliated with the *Gammaproteobacteria* (Simmons *et al.*, 2004). However, some doubt has been raised regarding the true phylogenetic relationship of this organism (Amann *et al.*, 2006) and the recent isolation of two new gammaproteobacterial rod-shaped MTB (Lefèvre *et al.*, 2010b) suggests that the organism described by Simmons *et al.* (2004) biomineralizes magnetite. Confirmed greigite-producing MTB whose 16S rRNA gene has been sequenced include only the multicellular magnetotactic prokaryote *Candidatus* Magnetoglobus multicellularis and bacteria morphologically similar to it (Abreu *et al.*, 2007; Simmons and Edwards, 2007). This microorganism consists of an assemblage of genetically identical, Gram-negative bacteria that are capable of collectively migrating along magnetic field lines because of the coordinated rotation of flagella that cover each cell on one side. Individual cells of this microorganism do not move or respond in a magnetic field. *Ca.* M. multicellularis is characterized by a multicellular life cycle with no apparent cell differentiation and intercellular communication between cells (Abreu *et al.*, 2007).

Correspondence: U Lins, Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, Rio de Janeiro 21941-902, Brazil.
E-mail: ulins@micro.ufrj.br
Received 25 October 2010; revised 15 February 2011; accepted 21 February 2011; published online 21 April 2011

The first report about greigite-producing MTB and its affiliation to *Deltaproteobacteria* suggest, but do not confirm, that magnetotaxis based on iron oxide and iron sulfide magnetosomes evolved independently and that the trait was polyphyletic (DeLong *et al.*, 1993).

Magnetite magnetosome formation in the *Alphaproteobacteria* has been extensively characterized in some cultured species (Jogler and Schüler, 2009) and briefly studied in uncultured organisms from environmental samples (Jogler *et al.*, 2009b). The genes responsible for magnetite biomineralization, the *mam* and *mms* genes, make up the *mamAB*, *mamGFDC*, *mamXY* and *mms6* operons (Jogler and Schüler, 2009) in a genomic island known as the magnetosome island (MAI; Schüler, 2004). These genes are responsible for controlling the size and morphology of magnetite crystals in MTB, as well as magnetosome chain organization (Schüler, 2004). Comparisons between the MAIs of different cultured magnetite-producing MTB show that gene content and organization differ among them and are thought to be responsible for differences in magnetosome crystal morphology and size and magnetosome organization (Jogler *et al.*, 2009a).

The ability of magnetosome synthesis is thought to have been distributed between these organisms by horizontal gene transfer of the MAI (Jogler and Schüler, 2009). According to a current model (Jogler *et al.*, 2009a), magnetite magnetosome genes were acquired by the magnetospirilla, the coccus *Candidatus* Magnetococcus marinus strain MC-1 (Schübbe *et al.*, 2009) and the vibrio *Candidatus* Magnetovibrio blakemorei strain MV-1 (Schübbe *et al.*, 2009) through independent events of horizontal gene transfer from an unknown ancestor. The close phylogenetic affinity of the magnetospirilla to a genus of photosynthetic bacteria, *Phaeospirillum*, suggests that the ancestor of these two groups of prokaryotes might have been a phototrophic organism. The absence of selective pressure for magnetotaxis and the loss of the MAI might have led to the occurrence of non-magnetotactic representatives within the MTB (Jogler *et al.*, 2009a). This model, however, describes magnetotaxis evolution only within the *Alphaproteobacteria* and considers only genes related to magnetite biomineralization. Genes for greigite magnetosome formation have not yet been found until now, so they have not been considered in this evolution model of magnetotaxis.

In this work, we show that genes related to magnetite biomineralization are conserved in greigite-producing MTB, suggesting that similar genes are involved in the biomineralization of both magnetite and greigite, but also that biomineralization of magnetite and greigite might not have evolved independently (that is, magnetotaxis is polyphyletic) as once suggested (DeLong *et al.*, 1993). Results presented here are consistent with a model in which the ability to biomineralize magnetosomes and the possession of the *mam* genes were possibly acquired by bacteria of the *Nitrospirae* and certainly acquired by *Proteobacteria* from an ancient common ancestor in independents horizontal gene transfer events, that is, the magnetotactic trait is monophyletic. This hypothesis is supported by the fact that *Ca.* M. multicellularis *mam* genes, when analyzed individually, are more related to orthologues found in both *Deltaproteobacteria* and also *Alphaproteobacteria* MTB and, when analyzed together, they are recovered as a deep branching lineage within bacteria.

Materials and methods

Sampling and sequencing

Because *Ca.* M. multicellularis has not been cultured, DNA samples were prepared according to a modified magnetic enrichment procedure (Lins *et al.*, 2003) which is based on capillary racetrack principle (Wolfe *et al.*, 1987). Water and sediment were collected from Araruama Lagoon, Rio de Janeiro, Brazil (22° 50′S, 42° 13′W) and magnetic enrichment was carried out with additional washing steps using lagoon sterile autoclaved water. This procedure yielded a highly concentrated sample of *Ca.* M. multicellularis. DNA extraction was performed (Chen and Kuo, 1993) and genomic DNA was amplified using REPLI-g mini kit (Qiagen, Hilden, Germany). *Ca.* M. multicellularis DNA was sequenced on 454 GS FLX System sequencer (Roche Diagnostics GmbH/454 Life Sciences Corporation, Branford, CT, USA). The purity of the *Ca.* M. multicellularis cell sample for DNA extraction was checked using light and electron microscopy on Zeiss Axiostar Plus microscope (Carl Zeiss, Oberkochen, Germany) and FEI Morgagni transmission electron microscope (FEI Company, Eindhoven, The Netherlands). The detection of *Ca.* M. multicellularis was greatly simplified by its unique morphology and the presence of magnetosomes (Supplementary Figure 1). No unicellular bacterium with or without magnetosomes was observed. The purity of the samples was also checked by amplification and sequencing of *16S rRNA* genes according to Abreu *et al.* (2007). The only 16S rRNA gene obtained from MTB was that of *Ca.* M. multicellularis.

Comparative analysis of magnetosome genes

The system for automated bacterial integrated annotation platform (Almeida *et al.*, 2004) was used to predict open reading frames (ORFs) position and sequence analysis using a tblastx tool (Altschul *et al.*, 1997). MAI genes of *M. magneticum* AMB-1 (AP007255), *M. gryphiswaldense* MRS-1 (AM085146), *M. magnetotacticum* MS-1 (NZ_AAAP01003731), *Ca.* Magnetococcus marinus strain MC-1 (NC_008576), *Ca.* Magnetovibrio blakemorei strain MV-1 (FP102531) and *Desulfovibrio magneticus* (AP010904) were compared with *Ca.* M. multicellularis sequences using tblastx for identity, positives and *e*-value analysis

(Altschul *et al*., 1997). Contigs having valid similarity values (*e*-value <1e-05) with *mam* genes were completely analyzed. Similar sequences of each MTB were compared between them for *e*-value determination. These sequences have been submitted to the GenBank databases under accession numbers HQ336745 and HQ336746.

Phylogenetic and bioinformatic analyses

Maximum likelihood trees based on 16S rRNA gene sequences and amino acid composition of *mam* genes were constructed although, for this study, phylogenetic analysis of Mam protein amino acid sequences was favored because sequences from distantly related taxa were analyzed (Opperdoes, 2009). Sequence alignment was performed with Muscle (version 3.6; Edgar, 2004). The general time-reversible model (GTR; Yang, 1994; Zharkikh, 1994) with gamma-distributed substitution rates was selected as the DNA substitution model by Modelgenerator (version 0.84; Keane *et al*. 2006). The Bayesian information criterion was used for the phylogenetic reconstruction. A maximum likelihood tree was then reconstructed using PhyML (version 3.0; Guindon and Gascuel, 2003). The tree topology space was explored using the nearest neighbor interchange and subtree pruning and regrafting algorithms starting from five random starting trees generated by BioNJ (Guindon and Gascuel, 2003; Guindon *et al*., 2010). Branch support was calculated using the approximate likelihood ratio test (aLRT) with SH-like interpretation. This approach is as conservative and accurate as bootstrapping but is less computationally intensive (Anisimova and Gascuel, 2006; Guindon *et al*., 2010).

Results

Partial genome sequencing of *Ca.* M. multicellularis and comparative analysis based on known *mam* and *mms* genes revealed the following orthologues (Table 1): *mamA*, *mamB*, *mamE*, *mamK*, *mamM*, *mamO*, *mamP*, *mamQ* and *mamT*. These genes were found in two segments of DNA (contigs) shown in Figure 1. Contig 1 (GenBank accession number HQ336746) is 15 950 bp long and consists of 754 reads (20 × coverage) whereas contig 2 (GenBank accession number HQ336745) is 9131 bp long and consists of 618 reads (30 × coverage), confirming data reliability and accuracy. Besides putative *mam* genes, contig 1 contains ORFs encoding for other genes possibly involved in magnetosome synthesis including an iron-dependent repressor, a putative ferrous iron transporter and several hypothetical proteins. The tblastx analysis of the first two sequences showed that they are similar to a putative iron repressor (*e*-value 7e-49; coverage 86%; identity 55%; positives 74%) and ferrous iron transporter found in the MAI (*e*-value 6e-110; coverage 85%;

Table 1 Comparison of magnetosome genes identified in *Candidatus* Magnetoglobus multicellularis with those of cultivated magnetotactic bacteria

Gene	*Desulfovibrio magneticus* RS-1		*Magnetospirillum magneticum* AMB-1		*Magnetospirillum magnetotacticum* MS-1		*Magnetospirillum gryphiswaldense* MSR-1		*Candidatus* Magnetococcus marinus MC-1		*Candidatus* Magnetovibrio blakemoreii MV-1	
	Coverage (%)	*ID/+(%)*	*Coverage (%)*	*ID/+(%)*	*Coverage (%)*	*ID/+(%)*	*Coverage (%)*	*ID/+(%)*	*Coverage (%)*	*ID/+(%)*	*Coverage (%)*	*ID/+(%)*
Magnetosome protein (*MamA)*	81	29/56	—	—	—	—	—	—	46	24/56	51	28/56
Magnetosome protein (*MamB)*	85	35/57	82	34/57	82	34/57	83	35/56	79	39/61	90	34/59
Magnetosome protein (*MamE)*	69	42/66	57	49/72	57	49/72	65	50/72	73	48/70	67	46/73
Magnetosome protein (*MamK)*	90	61/79	90	35/57	84	35/57	90	35/57	89	46/66	90	37/60
Magnetosome protein *(MamM)*	—	—	—	—	43	21/50	—	—	53	24/53	57	29/51
Magnetosome protein (*MamO)*	75	39/55	66	32/53	48	32/53	54	30/51	46	33/63	40	35/58
Hypothetical protein (*MamP*)*	69	39/62	—	—	37	40/65	—	—	—	—	—	—
Hypothetical protein (*MamP)*	68	32/55	—	—	—	—	—	—	22	27/51	—	—
Magnetosome protein (*MamQ)*	30	42/57	—	—	—	—	—	—	69	41/64	52	35/60
Magnetosome protein (*MamT)*	48	29/49	—	—	—	—	—	—	—	—	—	—

Accession numbers: *M. magneticum* AMB-1, AP007255; *M. gryphyswaldense* MSR-1, AM085146; *M. magnetotacticum* MS-1, NZ_AAAP01003731; *Ca.* M. marinus, NC_008576; *Ca.* M. blakemorei MV-1, FP102531; and *D. magneticus* RS-1, AP010904. The *e*-values above >1e-05 are not considered (−). ID/+ (%) represent identity and positives values in %.

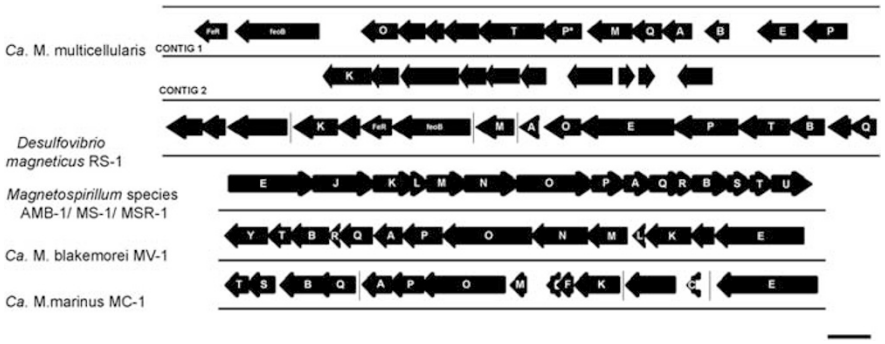

Figure 1 Organization of open reading frames in two segments of DNA (contigs) from *Candidatus* Magnetoglobus multicellularis containing putative magnetosome-related genes compared to the organization of magnetosome genes within the magnetosome gene island of several cultivated magnetotactic bacteria. Scale bar indicates 1000 bp. Hypothetical proteins are represented as unlabeled arrows.

identity 52%; positives 73%) of the magnetite-producing deltaproteobacterium *Desulfovibrio magneticus* (Nakazawa *et al.*, 2009). The position of these genes is also similar to *D. magneticus* as they are located upstream of *mam* genes. ORFs similar to an ATPase domain protein (2e-103; coverage 96%; identity 42%; positives 60%) and a hypothetical protein (*e*-value 8e-71; coverage 73%; identity 50%; positives 68%) found in *D. magneticus* were also present.

The G + C content of the two contigs containing *mam* genes is higher than the contig containing the *rrs* gene of *Ca.* M. multicellularis and putative genes involving metabolic pathways, possibly indicating that this genomic region is part of a MAI acquired by horizontal gene transfer from other organisms as described for magnetite-producing MTB (Jogler *et al.*, 2009a, b). The conserved genes *mamA*, *mamB*, *mamE*, *mamO* and *mamP*, *mamQ* and *mamT* appear to be organized in an operon; these genes are most likely essential for greigite magnetosome formation, as they may participate in the assembly of multi-protein complexes for magnetosome formation, iron transport and magnetosome organization (Jogler and Schüler, 2009). Interestingly, *Ca.* M. multicellularis contains two ORFs similar to the *mamP* gene. However, these sequences are more similar to each other (*e*-value 7e-19; coverage 61%; identities 34%; positives 59%) than to *mamP* from other MTB.

The *mms6*, *mamD*, *mamC*, *mamF*, *mamG*, *mamJ*, *mamX* and *mamY* genes, strongly conserved in most magnetite-producing magnetotactic *Alphaproteobacteria* (Jogler *et al.*, 2009a), were not found in our sequences or in the genome of *D. magneticus* (Nakazawa *et al.*, 2009). These genes are considered to be of great importance in controlling magnetosome size and morphology in MTB of the *Alphaproteobacteria*. Nakazawa *et al.* (2009) suggested that their absence in *D. magneticus* might explain the presence of irregular, bullet-shaped magnetosomes in this species rather than the consistent hexa- and octahedra produced by *Alphaproteobacteria* MTB that possess these genes. Greigite magnetosome crystals, such as the bullet-shaped magnetite crystals, are pleomorphic (Abreu *et al.*, 2007), which might be an indication that orthologues of these genes are not present in MTB that biomineralize greigite and bullet-shaped magnetite crystals. Moreover, some greigite-producing MTB biomineralize bullet-shaped magnetite crystals in addition to greigite (Bazylinski *et al.*, 1993). Our results suggest that the minimum magnetosome-related genes required for the synthesis of magnetite or greigite magnetosomes include *mamA*, *mamB*, *mamE*, *mamK*, *mamM*, *mamO*, *mamP*, *mamQ* and *mamT*, which are shared among MTB of both the *Alphaproteobacteria* and *Deltaproteobacteria*.

The highest similarity values according to tblastx analysis for ORFs from the contigs of *Ca.* M. multicellularis were for *mamA* (*e*-value 2e-25), *mamB* (*e*-value 3e-62), *mamK* (*e*-value 8e-137), *mamO* (*e*-value 1e-49), *mamP** (*e*-value 7e-11), *mamP* (*e*-value 3e-19) and *mamT* (*e*-value 8e-08), all from *D. magneticus*, whereas *mamE* (*e*-value 1e-50) and *mamQ* (*e*-value 2e-18) were more similar to those of *Ca.* Magnetococcus marinus and *mamM* (*e*-value 1e-14) to that of *Ca.* Magnetovibrio blakemorei strain MV-1. *Ca.* M. multicellularis Mam amino acid sequences, when analyzed together, are more similar to those of *D. magneticus*, which is in accordance with the phylogenetic analysis based on 16S rRNA gene sequences (Figures 1 and 2). Phylogenetic analysis based on Mam amino acids sequences suggests that a more recent horizontal gene transfer occurred from *Ca.* M. multicellularis and *D. magneticus* than the other species, but they are still distantly related (Figure 2).

Discussion

The relatively high similarity values found between the *mam* orthologues in *Ca.* M. multicellularis and the *mam* genes of MTB of both the *Alphaproteobacteria* (*mam*E, *mam*M and *mam*Q) and *Deltaproteobacteria* (*mam*A, *mam*B, *mam*K, *mam*O, *mam*P and *mam*T) suggests that these genes evolved from a common ancestor. Moreover, phylogenetic relationships of MTB based on Mam amino acid sequences and on 16S rRNA gene sequences suggest that

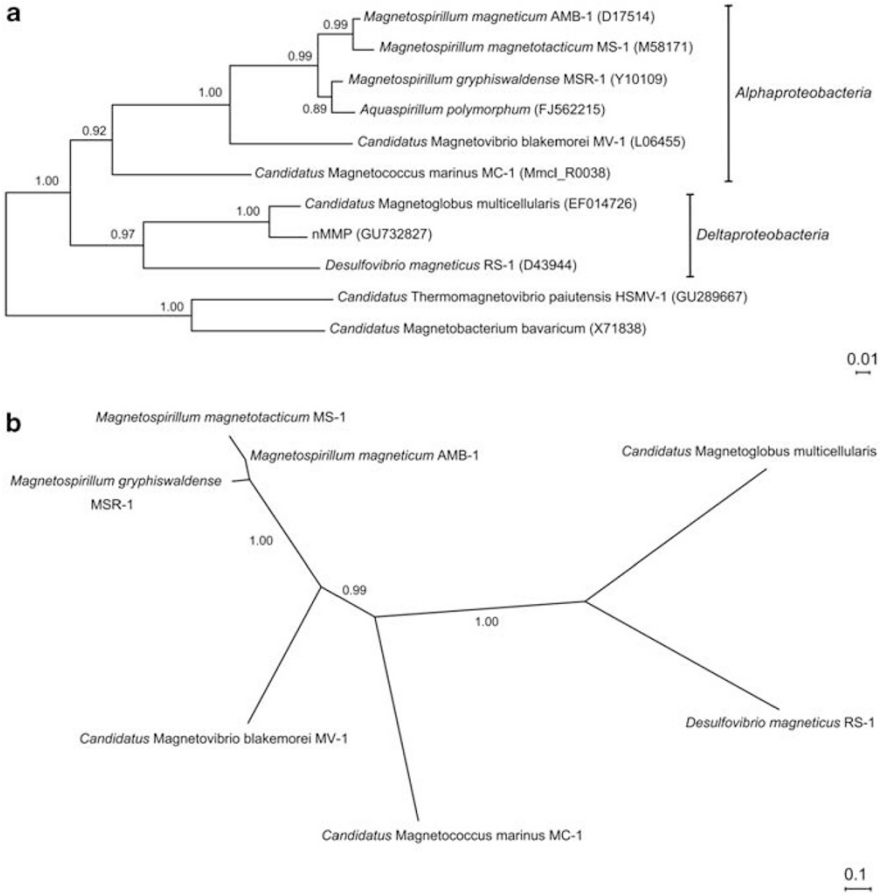

Figure 2 Phylogenetic analyses of *Candidatus* Magnetoglobus multicellularis: (**a**) maximum likelihood tree based on 16S rRNA gene sequences using the GTR + Γ DNA substitution model. Numbers at nodes represent bootstrap values for 1000 replicates. Numbers in parentheses are GenBank accession numbers. (**b**) Amino acid composition of conserved *mam* genes using the WAG + I + Γ + F matrix. Sequences of the uncultured MTB *C.* Thermomagnetovibrio paiutensis HSMV-1 (Lefèvre *et al.*, 2010a) and *Ca.* Magnetobacterium bavaricum (Spring *et al.*, 1993), both phylogenetically affiliated with the *Nitrospirae* phylum, are included as outgroups in this analysis. Numbers between branches are aLRT values.

magnetite and greigite biomineralization did not evolve independently, at least in the *Proteobacteria*. Phylogenetic analysis based on Mam protein amino acids sequences and *mam* genes content suggest that *D. magneticus* and *Ca.* M. multicellularis might share an unknown magnetotactic deltaproteobacterial ancestor. According to the prokaryotic evolution point of view (Gogarten *et al.*, 2002), frequency of successful exchange between taxa will depend on specific factors, including propinquity, metabolic compatibility, adaptation to the abiotic environment, gene expression systems and gene-transfer mechanisms. Based on this, acquisition of *mam* genes by horizontal gene transfer between *D. magneticus* and *Ca.* M. multicellularis might have a high probability as they both inhabit anoxic sulfide-rich environments below the oxic–anoxic transition zone, unlike almost all other magnetite-producing MTB.

A number of magnetosome-related genes, including *mam*A, *mam*B, *mam*E, *mam*I, *mam*M, *mam*P and *mam*Q, were recently found to be present in the genome of *Candidatus* Magnetobacterium bavaricum, a magnetite-producing MTB phylogenetically affiliated with the *Nitrospirae* phylum (Jogler *et al.*, 2011). These genes not only show similar sequence homologies to others of their type, the organization of these genes show similar short intergenic distances between them and an identical direction of transcription, providing evidence that they are organized as an operon as described for other MTB (Schübbe *et al.*, 2006). The presence of *mam* genes and their organization in the *Nitrospirae* and various classes of the *Proteobacteria* strongly supports a monophyletic origin for magnetite-based magnetotaxis. Because both the greigite-producing *Ca.* M. multicellularis and the magnetite-producing MTB share similar *mam* genes, the capability of magnetosome, regardless of whether they contain iron oxide or iron sulfide crystals, synthesis in all currently known MTB appears to be a result of the acquisition of *mam* genes by independent horizontal gene transfer events from a common ancestor during evolution.

This scenario raises a number of interesting and important questions. What was the first magnetic mineral biomineralized by the MTB? It seems from the information presented here that it was likely

magnetite, as MTB from the most deeply branching groups of *Bacteria* (Emerson *et al.*, 2007) that contain them, the *Nitrospirae* and the *Deltaproteobacteria* produce this mineral. Moreover, MTB from these groups are known to biomineralize only bullet-shaped magnetite crystals suggesting that this crystal morphology is the earliest form of magnetosome magnetite. If true, this finding has important implications in the finding and interpretation of magnetofossils, the putative remains of MTB magnetite crystals in ancient and recent sediments (Jimenez-Lopez *et al.*, 2010). It is unclear if any of the *mam* genes are involved in the selective precipitation of magnetite or greigite in magnetosomes. Greigite formation may have been a modification of magnetite biomineralization in some sulfate-reducing MTB such as *Ca.* M. multicellularis under certain conditions. This possibility is supported by the fact that the positioning of *Ca.* M. multicellularis in sediment is influenced by the physicochemical properties of the microenvironment, for example, the redox potential (Eh) and iron/sulfur availability (Sobrinho *et al.*, 2011). According to Sobrinho *et al.* (2011) *Ca.* M. multicellularis is positioned where the Eh and pH facilitates the formation of iron monosulfides, a condition necessary for the formation of greigite in magnetosomes (Pósfai *et al.*, 1998b). In addition, environmental conditions appear to influence the magnetosome mineral composition in some greigite-producing rod-shaped bacteria that also produce magnetite (Bazylinski *et al.*, 1995).

More recently diverging groups of the *Proteobacteria* (Emerson *et al.*, 2007), the *Alpha-* and *Gammaproteobacteria*, that contain MTB that biomineralize cubooctahedral and elongated prismatic crystal morphologies of magnetite (Bazylinski and Frankel, 2004; Lefèvre *et al.*, 2010b) and possess more *mam* genes, might have acquired this ability through gene mutation and gene duplication or acquisition of additional *mam*-like genes that were later distributed between species of these groups by horizontal gene transfer. The presence of *mam* genes may not be sufficient to explain all the differences in morphology and mineral content of magnetosomes; the presence of a membrane enveloping the crystals appears to be essential to the biomineralization of the consistent, regularly-shaped magnetosome morphologies (for example, hexahedral prisms) observed in some species. Our data show that the *mam* gene content of *D. magneticus* and *Ca.* M. multicellularis is similar but these microorganisms appear to be distinct in the presence/absence of a membrane enveloping the crystals and in their magnetosome crystal morphologies. The absence of a magnetosome membrane in *D. magneticus* (Byrne *et al.*, 2010) may explain the irregular morphologies of magnetite formed by this microorganism despite the presence of *mam* genes in its genome (Nakazawa *et al.*, 2009). *Ca.* M. multicellularis magnetosomes are enveloped by a membrane (Abreu *et al.*, 2008) similar to those present in some *Nitrospirae* (Lefèvre *et al.*, 2011) and in magnetotactic multicellular prokaryotes capable of simultaneous biomineralization of magnetite and greigite magnetosomes (Lins *et al.*, 2007); these uncultured microorganisms produce regularly shaped magnetosomes. It is also possible that the process of magnetite biomineralization used by *D. magneticus* is different under the culture conditions used to grow this organism compared with what occurs in nature. Further investigation of the physiology of divergent MTB species is needed to achieve a deeper understanding of the differences between magnetite and greigite biomineralization in MTB.

Acknowledgements

The financial support from the Brazilian agencies, CNPq, CAPES and FAPERJ is acknowledged. CTL and DAB were supported by the US National Science Foundation grant EAR-0920718.

References

Abreu F, Martins JL, Silveira TS, Keim CN, Lins de Barros HG P, Gueiros-Filho F *et al.* (2007). '*Candidatus* Magnetoglobus multicellularis' a multicellular magnetotactic prokaryote from a hypersaline environment. *Int J Syst Bacteriol* **57**: 1318–1322.

Abreu F, Silva KT, Farina M, Keim C, Lins U. (2008). Greigite magnetosome membrane ultrastructure in *Candidatus* Magnetoglobus multicellularis. *Int Microbiol* **11**: 75–80.

Almeida LG, Paixão R, Souza RC, Costa GC, Barrientos FJ, Santos MT *et al.* (2004). A system for automated bacterial (genome) integrated annotation—SABIA. *Bioinformatics* **20**: 2832–2833.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W *et al.* (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 389–3402.

Amann R, Peplies J, Schüler D. (2006). Diversity and taxonomy of magnetotactic bacteria. In: Schüler D (ed). *Magnetoreception and Magnetosomes in Bacteria*. Springer: Berlin, Germany, pp 25–36.

Anisimova M, Gascuel O. (2006). Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst Biol* **55**: 539–552.

Bazylinski DA, Frankel RB. (2004). Magnetosome formation in prokaryotes. *Nat Rev Microbiol* **2**: 217–230.

Bazylinski DA, Frankel RB, Heywood BR, Mann S, King JW, Donaghay PL *et al.* (1995). Controlled biomineralization of magnetite (Fe_3O_4) and greigite (Fe_3S_4) in a magnetotactic bacterium. *Appl Environ Microbiol* **61**: 3232–3239.

Bazylinski DA, Heywood BR, Mann S, Frankel RB. (1993). Fe_3O_4 and Fe_3S_4 in a bacterium. *Nature* **366**: 218.

Byrne ME, Ball DA, Guerquin-Kern JL, Rouiller I, Wu TD, Downing KH *et al.* (2010). *Desulfovibrio magneticus* RS-1 contains an iron- and phosphorus-rich organelle distinct from its bullet-shaped magnetosomes. *Proc Natl Acad Sci USA* **107**: 12263–12268.

Chen W, Kuo T. (1993). A simple and rapid method for the preparation of Gram-negative bacterial genomic DNA. *Nucleic Acids Res* **21**: 2260.

DeLong EF, Frankel RB, Bazylinski DA. (1993). Multiple evolutionary origins of magnetotaxis in bacteria. *Science* **259**: 803–806.

Edgar RC. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**: 1792–1797.

Emerson D, Rentz JA, Lilburn TG, Davis RE, Aldrich H, Chan C *et al.* (2007). A novel lineage of proteobacteria involved in formation of marine Fe-oxidizing microbial mat communities. *PLoS ONE* **8**: e667.

Gogarten JP, Doolittle WF, Lawrence JG. (2002). Prokaryotic evolution in light of gene transfer. *Mol Biol Evol* **19**: 2226–2238.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* **59**: 307–321.

Guindon S, Gascuel O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**: 696–704.

Jimenez-Lopez C, Romanek CS, Bazylinski DA. (2010). Magnetite as a prokaryotic biomarker: a review. *J Geophys Res-Biogeo* **115**: G00G03.

Jogler C, Kube M, Schübbe S, Ullrich S, Teeling H, Bazylinski DA *et al.* (2009a). Comparative analysis of magnetosome gene clusters in magnetotactic bacteria provides further evidence for horizontal gene transfer. *Environ Microbiol* **15**: 1267–1277.

Jogler C, Lin W, Meyerdierks A, Kube M, Katzmann E, Flies C *et al.* (2009b). Toward cloning of the magnetotactic metagenome: identification of magnetosome island gene clusters in uncultivated magnetotactic bacteria from different aquatic sediments. *Appl Environ Microbiol* **75**: 3972–3979.

Jogler C, Schüler D. (2009). Genomics, genetics, and cell biology of magnetosome formation. *Annu Rev Microbiol* **63**: 501–521.

Jogler C, Wanner G, Kolinko S, Nieber M, Amann R, Petersen N *et al.* (2011). Conservation of proteobacterial magnetosome genes and structures in an uncultivated member of the deep-branching *Nitrospira* phylum. *Proc Natl Acad Sci USA* **108**: 1134–1139.

Keane TM, Creevey CJ, Pentony MM, Naughton TJ, McLnerney JO. (2006). Assessment of methods for amino acid matrix selection and their use on empirical data shows that *ad hoc* assumptions for choice of matrix are not justified. *BMC Evol Biol* **6**: 29.

Lefèvre CT, Abreu F, Schmidt ML, Lins U, Frankel RB, Hedlund BP *et al.* (2010a). Moderately thermophilic magnetotactic bacteria from hot springs in Nevada. *Appl Environ Microbiol* **76**: 3740–3743.

Lefèvre CT, Frankel RB, Abreu F, Lins U, Bazylinski DA. (2011). Culture-independent characterization of a novel, uncultivated magnetotactic member of the *Nitrospirae* phylum. *Environ Microbiol* **13**: 538–549.

Lefèvre CT, Viloria N, Abreu F, Pósfai M, Prozorov T, Lins U *et al.* (2010b). Phylogenetic diversity and ecophysiology of new cultured and uncultured magnetite-producing magnetotactic bacteria from aquatic environments in the desert. Abstract, *2nd International Symposium on Magnetotactic Bacteria and Biomineralization*, Beijing, China. pp 34–36.

Lins U, Freitas F, Keim CN, Lins de Barros HGP, Esquivel DSM, Farina M. (2003). Simple homemade apparatus for harvesting uncultured magnetotactic microorganisms. *Braz J Microbiol* **34**: 111–116.

Lins U, Keim CN, Evans FF, Farina M, Buseck PR. (2007). Magnetite (Fe_3O_4) and greigite (Fe_3S_4) crystals in multicellular magnetotactic prokaryotes. *Geomicrobiol J* **24**: 43–50.

Nakazawa H, Arakaki A, Narita-Yamada S, Yashiro I, Jinno K, Aoki N *et al.* (2009). Whole genome sequence of *Desulfovibrio magneticus* strain RS-1 revealed common gene clusters in magnetotactic bacteria. *Genome Res* **19**: 1801–1808.

Opperdoes FR. (2009). Phylogenetic analysis using protein sequences. In: Lemey P, Salemi M, Vandamme A-M (eds). *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis Testing*. Cambridge University Press: Cambridge, pp 313–331.

Pósfai M, Buseck PR, Bazylinski DA, Frankel RB. (1998a). Iron sulfides from magnetotactic bacteria: structure, composition, and phase transitions. *Am Mineral* **83**: 1469–1481.

Pósfai M, Buseck PR, Bazylinski DA, Frankel RB. (1998b). Reaction sequence of iron sulfide minerals in bacteria and their use as biomarkers. *Science* **280**: 880–883.

Schübbe S, Williams TJ, Xie G, Kiss HE, Brettin TS, Martinez D *et al.* (2009). Complete genome sequence of the chemolithoautotrophic marine magnetotactic coccus strain MC-1. *Appl Environ Microbiol* **75**: 4835–4852.

Schübbe S, Würdemann C, Peplies J, Heyen U, Wawer C, Glöckner FO *et al.* (2006). Transcriptional organization and regulation of magnetosome operons in *Magnetospirillum gryphiswaldense*. *Appl Environ Microbiol* **72**: 5757–5765.

Schüler D. (2004). Molecular analysis of a subcellular compartment: the magnetosome membrane in *Magnetospirillum gryphiswaldense*. *Arch Microbiol* **181**: 1–7.

Simmons SL, Bazylinski DA, Edwards KJ. (2004). Spatiotemporal distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond. *Appl Environ Microbiol* **70**: 6230–6239.

Simmons SL, Edwards KJ. (2007). Unexpected diversity in populations of the many-celled magnetotactic prokaryote. *Environ Microbiol* **9**: 206–215.

Sobrinho R, Lins U, Bernardes M. (2011). Geochemical characteristics related to the greigite-producing multicellular magnetotactic prokaryote '*Candidatus* Magnetoglobus multicellularis' in a hypersaline lagoon. *Geomicrobiol J* (in press).

Spring S, Amann R, Ludwig W, Schleifer KH, van Gemerden H, Petersen N. (1993). Dominating role of an unusual magnetotactic bacterium in the microaerobic zone of freshwater sediment. *Appl Environ Microbiol* **59**: 2397–2403.

Wolfe RS, Thauer RK, Pfennig N. (1987). A 'capillary racetrack' method for isolation of magnetotactic bacteria. *FEMS Microbiol Ecol* **45**: 31–35.

Yang Z. (1994). Estimating the pattern of nucleotide substitution. *J Mol Evol* **39**: 105–111.

Zharkikh A. (1994). Estimation of evolutionary distances between nucleotide sequences. *J Mol Evol* **39**: 315–329.

Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)